Dynamics of glycolipids in the liquid-crystalline state
^{2}H NMR study

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ABSTRACT It has been shown previously that two types of motion are adequate to describe the partially relaxed ^{2}H NMR line shapes (inversion recovery experiment) for the backbone portion of the glycolipid 1,2-di-*O*-tetradecyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerol (β-DTGL) in the highly ordered gel phase (Auger, M. A., D. Carrier, I. C. P. Smith, and H. C. Jarrell. 1990. *J. Am. Chem. Soc.* 112:1373–1381). This study extends the latter investigation to the more fluid liquid-crystalline phase, where more complex motions are anticipated. Analyses of the powder line shapes and oriented sample relaxation data for both the glycerol backbone and head group regions of this lipid have been performed. The dynamics of glycerol at the C3 position in the gel state have been described by large angle jumps about the C2-C3 bond with a correlation time in the fast-limit motional regime ($\omega_o\tau_c \ll 1$) and site populations 0.46, 0.34, and 0.20. The present data show that in the liquid-crystalline phase the internal jump rate is maintained, and two additional motions are necessary to describe the dependence of the relaxation rate on the orientation of the director with respect to the magnetic field direction. These are rotation about the molecular long axis with a correlation time in the slow-limit motional regime very near to the T_1 minimum ($\omega_o\tau_c \approx 0.65$), and molecular fluctuations about the order director (modeled by a Maier–Saupe restoration potential). This treatment was also extended to the glucose head group where additional segmental motion about the glycosidic bond has been reported previously. While the two motions dominating relaxation at the glycerol C3 segment reproduce the general relaxation features of the glucose head group, the results suggest that additional motion about the glycosidic linkage must be present. This study is a stringent test of the motional model chosen earlier because relaxation data were obtained at two ^{2}H NMR frequencies using two relaxation experiments (T_{1Z} and T_{1Q}) and two types of sample preparation (oriented and dispersed multibilayers). The results strongly uphold the choice of model and indicate the utility of both oriented samples and the T_{1Q} experiment.

INTRODUCTION

Cell surface carbohydrates play a crucial role in cellular biology. Glycolipids have been implicated in important cellular events such as cell–cell recognition (Blackburn et al., 1986), ligand–receptor interactions (Hakomori, 1986), and as modulators of membrane structure (Curatolo, 1987). The accessibility of glycolipids to such interactions is governed in part by two properties: head group conformation/orientation at the membrane surface, and lipid dynamics. Conformation/orientation studies probe the spatial distributions of these molecules, and dynamic studies involve the investigation of the types and rates of motions present in a given system. Deuterium (^{2}H) NMR has proven to be an extremely valuable tool in studying both orientation and dynamics of ordered environments, such as model membrane systems (Seelig, 1977; Seelig and Seelig, 1980; Davis, 1983; Smith, 1989). ^{2}H labeling essentially isolates the molecular segment of interest because the intramolecular ^{2}H quadrupolar interaction dominates the NMR spectrum (Abragam, 1961). As a result, the ^{2}H NMR spectrum is sensitive to local orientation and anisotropic motions (Seelig, 1977). This is significant because the time frame of the motions of interest lies within that of the experimental technique ($1–10^{10}\,s^{-1}$) (Abragam, 1961). Furthermore, the ability to measure two spin-lattice relaxation times (T_{1Z} and T_{1Q}) makes spin 1 nuclei (such as ^{2}H) potentially more powerful molecular probes than spin 1/2 nuclei (which have only one spin-lattice relaxation time, T_{1Z}) (Jacobsen et al., 1976; Jacobsen and Schaumburg, 1976).

In our recent studies of carbohydrate structure and dynamics much attention has been devoted to the glycolipid 1,2-di-*O*-tetradecyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerol (β-DTGL;[1] Jarrell et al., 1986, 1987*a*,*b*) (Fig. 1) and related compounds (Carrier et al., 1989; Renou et al., 1989). β-DTGL is a reasonable analogue of many biologically relevant glycosphingolipids where

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[1] *Abbreviations used in this paper:* DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DPPC, dipalmitoyl phosphatidylcholine; β-DTGL, 1,2-di-*O*-tetradecyl-3-*O*-(β-D-glucopyranosyl)-*sn*-glycerol.

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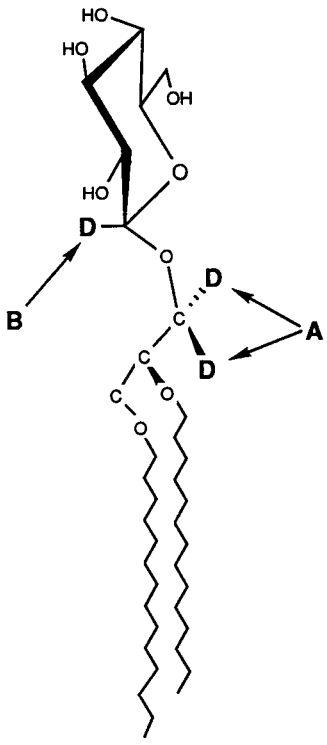

FIGURE 1 Diagrammatic representation of β-DTGL with approximate orientation along the bilayer normal. (*A*) Deuteration at the glycerol C3 position, [3,3-$^2H_2$] β-DTGL. (*B*) Deuteration at the 1′ position of glucose, [1′-$^2H_1$] β-DTGL.

the first sugar within the oligosaccharide head group is usually glucose.

While NMR relaxation studies can provide a wealth of information about the time scale of molecule motion, such measurements are performed with the goal of extending the physical/chemical description of the system under investigation. Therefore, to fully utilize the potential of such measurements, it is essential to analyze the results within the context of an adequate motional model. Previous work from this laboratory concluded that for β-DTGL in the gel phase two motions were manifest at the glycerol *sn*-3 position. The motion affecting relaxation is in the fast-limit motional regime ($\tau_c = 6.7 \times 10^{-10}$ s; $\omega_o\tau_c \ll 1$) and consists of rotation about the glycerol C2-C3 bond with site populations 0.46, 0.34, and 0.20. A second, slower motion ($\tau_c > 3.0 \times 10^{-7}$ s, slow-limit; $\omega_o\tau_c \gg 1$) involved the rotation of the molecule as a whole about the molecular long axis (Auger et al., 1990).

It is expected that molecular motions will be less complicated and slower in the relatively highly ordered (segmental order parameter, $S_{mol} \approx 1$) gel phase than in the more biologically relevant and less ordered ($S_{mol} < 1$) liquid-crystalline (L_α) phase. Thus, in the case of β-DTGL it is of interest to explore whether the gel-state motional description can form the basis for an adequate interpretation of molecular reorientation in the L_α phase for both the glycerol ($S_{mol} = 0.65$) and the carbohydrate ($S_{mol} = 0.45$) regions of the lipid. (S_{mol} varies in value from 0 to 1 and is indicative of the degree of segmental order [see below]).

Essentially, we anticipate a stepwise progression in motional complexity when considering the dynamics of the glycerol backbone in the gel state, this region in the L_α phase, and then the L_α phase carbohydrate (head group) region. This progression is expected because the respective segmental order parameters (S_{mol}) at the glycerol backbone decrease upon the transition from gel to fluid L_α phase. In turn, the S_{mol} of the backbone region is greater than that of the head group region, which is an indication of more restricted motion than in the head group region.

This study rigorously tests the ability of the model used previously for the gel phase to describe dynamics in the liquid-crystalline phase of β-DTGL by using two ^2H NMR observation frequencies, two types of sample preparation (oriented and dispersed multibilayers), and two relaxation experiments (T_{1Z} and T_{1Q}). Frequency-dependent studies are an accepted approach to testing motional models in lipid systems (Brown, 1984; Dufourc and Smith, 1986). We shall further establish the importance of using oriented samples and the T_{1Q} experiment to obtain unambiguous information on the system of interest.

MATERIALS AND METHODS

Lipids and preparations

Dipalmitoylphosphatidylcholine (DPPC) was obtained from Sigma Chemical Co., St. Louis, MO. Both 1,2-di-*O*-tetradecyl-3-*O*-(β-D-glucopyranosyl)-*sn*-[3,3-$^2H_2$]glycerol ([3,3-$^2H_2$] β-DTGL) and 1,2-di-*O*-tetradecyl-3-*O*-(β-D-[1′-$^2H_1$]glucopyranosyl)-*sn*-glycerol ([1′-$^2H_1$] β-DTGL) were prepared as described previously (Jarrell et al., 1986, 1987*b*). Multilamellar dispersion samples for ^2H NMR consisted of 50–100 mg of dry lipid hydrated with a 10-fold excess of ^2H-depleted water (Aldrich Chemical Co., Milwaukee, WI) in 5- and 10-mm (o.d.) sample tubes. Hydrated samples were heated cyclically to 60°C with vortex mixing and freeze-thawed to homogeneity (four to five cycles). For the preparation of oriented samples, the required amount of each lipid (30–60 mg total lipid; DPPC/β-DTGL, 1:4 molar ratio) was dissolved in chloroform/methanol (2:1, vol/vol). The lipid mixture was dropped on 22 × 7 × 0.15-mm glass plates and allowed to dry. The plates were stacked in a 10-mm (o.d.) NMR tube and dried under vacuum for 3 h. The sample was hydrated overnight at 55°C in a saturated atmosphere of ^2H-depleted water. Afterwards, a drop of ^2H-depleted water was added and the tube was sealed (Jarrell et al., 1987*b*).

NMR spectroscopy

^{2}H NMR data were acquired at 46.1 MHz on an MSL-300 spectrometer (Bruker Instruments, Inc., Billerica, MA), with a 10-mm solenoidal sample coil ($\pi/2$ pulse = 4.0–4.5 μs), and at 30.7 MHz on a "home-built," solid-state spectrometer, using 7- and 10-mm solenoidal sample coils ($\pi/2$ pulses = 3.8 and 4.0–4.6 μs, respectively). Spin-lattice relaxation times T_{1Z} and T_{1Q} were obtained using the standard inversion–recovery sequence coupled with the quadrupolar echo sequence (Dufourc et al., 1984) and the Jeener–Broeckaert experiment with a refocusing 90° pulse (Jeffrey, 1981), respectively. Spectra were acquired with full phase cycling (Griffin, 1981) and with quadrature detection. Recycle times were greater than $5T_{1Z}$ and $5T_{1Q}$ for the respective experiments, and pulse spacing of the quadrupolar echo was typically 60 μs. All spectra were acquired in the liquid-crystalline (L_α) phase at 54.0 ± 1.0°C.

Computer simulations

Orientation-dependent relaxation profiles ($T_1(\beta)$) and partially relaxed inversion–recovery powder spectra were simulated on a model 4-260 computer (Sun Microsystems, Inc., Mountain View, CA) as described previously (Bonmatin et al., 1990) using a modified line shape program (Wittebort et al., 1987) which was based on the formalism of Torchia and Szabo (1982). In addition, the effects of partial orientational averaging of the T_1 anisotropy resulting from molecular fluctuations (β″, Fig. 2) was modeled within a Maier–Saupe ordering potential (Van De Ven and Levin, 1984; see below). A line shape simulation program (Perly et al., 1985) to calculate partially relaxed spectra was modified to include the above relaxation profiles into the line shape simulations. Simulated line shapes were corrected for the finite width of the pulses in the inversion–recovery experiment (Hiyama et al., 1986).

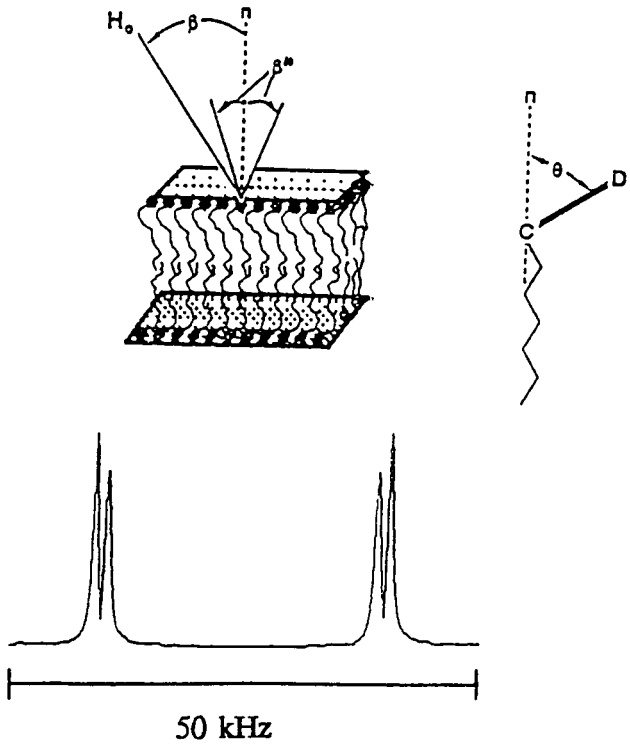

FIGURE 2 (*Top left*) Schematic diagram of oriented multibilayers; experimentally β is the angle between the bilayer normal (n) and the applied magnetic field (H_o) and β″ is representative of a distribution of molecular orientations about n (see below). (*Right*) Representation of angle (θ) between the C-^{2}H bond and n. (*Bottom*) Spectrum of L_α [3,3-$^2H_2$] β-DTGL oriented at 90°. The two quadrupolar splittings indicate that the two ^{2}H atoms on the glycerol C3 position are inequivalent, θ = 71.7 and 73.7° (Jarrell et al., 1987*b*).

RESULTS AND DISCUSSION

The glycerol backbone

In this study both oriented and randomly dispersed multibilayers of β-DTGL in the L_α phase were used. As will be noted, both types of preparation have significant uses in testing the model that will describe the motions and rates of the molecule. The oriented samples (Fig. 2) were physically rotated (angle β) and both T_{1Z} and T_{1Q} spin-lattice relaxation times were determined for a number of orientations. From these data, orientation-dependent ($T_1(\beta)$) relaxation profiles were constructed and T_1 anisotropies ($\partial T_1(\beta) = T_1(0) - T_1(90)$) were determined accurately. The class of motional model considered is one in which the C-^{2}H bond reorients about the symmetry axis at a fixed angle θ (Torchia and Szabo, 1982). In the L_α phase of [3,3-^{2}H] β-DTGL, the two ^{2}H atoms on the glycerol C3 position are inequivalent and two splittings can be observed (Fig. 2). The basis of this inequivalence is the angle of the C-^{2}H bond orientation (θ) with respect to the principle direction of ordering (order director), which has been calculated for both positions (73.7 and 72.5°) (Jarrell et al., 1987*b*). For the simulations the average orientation (θ = 73.2°) was used; there was no significant difference in the results of the simulations when θ was varied by ±1.5°.

Previous work on gel phase [3,3-$^2H_2$] β-DTGL described the dynamics at the glycerol C3 position using two motions: a fast, large angle jump among three sites about the glycerol C2-C3 bond (i.e., trans-gauche isomerization) and a slower axial motion of the molecule as a whole. It was concluded that this fast isomerization ($\tau_c = 6.7 \times 10^{-10}$ s, fast limit motional regime; $\omega_o\tau_c \ll 1$) among the three sites (populations 0.46, 0.34, and 0.20) dominated spin-lattice relaxation. The second motion was attributed to rotation about the molecular long axis. Addition of this motion to the simulations of the partially relaxed gel phase spectra did not alter the spin-lattice relaxation time, but was necessary to match simulated line shapes and quadrupolar echo intensities to spectra acquired over the temperature range 25–60°C. The rotation rate was varied in the range of the exchange minimum ($\omega_Q\tau_c \approx 1$, $\omega_Q = 2\pi\Delta\upsilon_Q$; $\Delta\upsilon_Q$ is the

quadrupolar splitting) where the line shape is most sensitive. The fastest axial rotation rate used ($\tau_c = 3.3 \times 10^{-7}$ s; fast-limit motional regime, $\omega_Q\tau_c \ll 1$) is fast enough to average the electric field gradient tensor to axial symmetry, but it is too slow to contribute to spin-lattice relaxation ($\omega_o\tau_c \gg 1$, slow-limit T_1 motional regime) (Abragam, 1961; Slichter, 1990).

The above parameters from the gel-state study were used as a starting point for the treatment of [3,3-$^2H_2$] β-DTGL in the L_α phase. The simulated and experimental $T_1(\beta)$ relaxation profiles for both T_{1Z} and T_{1Q} obtained at 30.7 MHz are given in Figs. 3 *A* (T_{1Z}) and 4 *A* (T_{1Q}) and at 46.1 MHz in Fig. 5, *A* (T_{1Z}) and *C* (T_{1Q}). In each of the four cases the relative trends in the anisotropies ($\partial T_1(\beta)$) of the simulated relaxation profiles are in agreement with the experiment, corroborating the findings of the gel-phase study. However, the experimental profiles are much less anisotropic than their simulated counterparts, suggesting that there are additional factors influencing the relaxation profiles. This is evident upon consideration of the partially relaxed T_{1Z} spectra (Fig. 6 *B* at 30.7 MHz; result not shown for 46.1 MHz); the experimental spectra appear to be more fully recovered than the simulated spectra.

The discrepancy between the simulated and experimental results is not surprising since the segmental order parameter for position C3 of the glycerol moiety of β-DTGL in the gel phase ($S_{mol} \approx 1$) is larger than that of

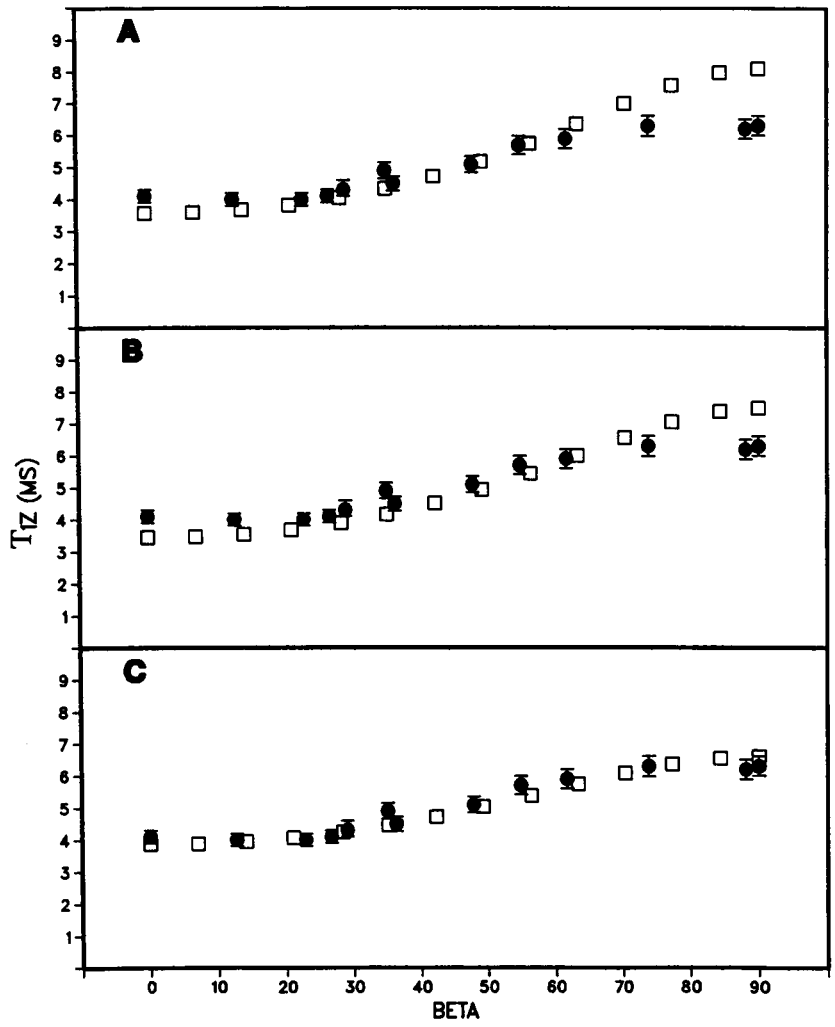

FIGURE 3 Angle-dependent T_{1Z} profiles, oriented multibilayers of [3,3-$^2H_2$] β-DTGL at 30.7 MHz. Experimental (●) and calculated (□) ^2H T_{1Z} spin-lattice relaxation times as a function of β (angle between H_o and bilayer normal). (*A*) Three-site jump ($\tau_c = 6.7*10^{-10}$ s; fast limit, $\omega_o\tau_c \ll 1$) with site populations 0.46, 0.34, and 0.20 plus axial rotation about the molecular long axis ($\tau_c = 3.0*10^{-7}$ s; slow limit, $\omega_o\tau_c \ll 1$). (*B*) As in *A*, but with increased axial rotation rate ($\tau_c = 8.3*10^{-9}$ s; close to T_1 minimum, $\omega_o\tau_c \approx 0.65$). (*C*) As in *B*, plus effects of Maier-Saupe averaging; S_{mol} = 0.65. Replication of data was reproducible within 2%. Experimental error was estimated to be ±5%. All data were acquired at 54°C.

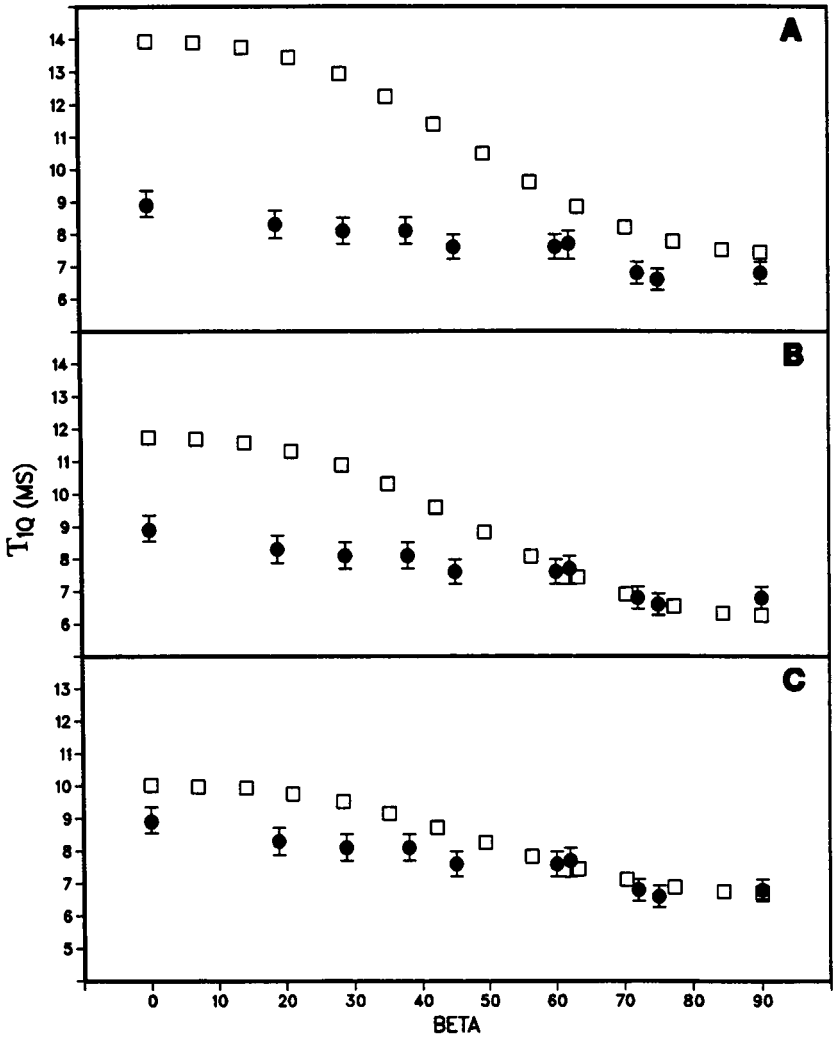

FIGURE 4 Angle-dependent T_{1Q} profiles, oriented multibilayers of {3,3-$^2H_2$} β-DTGL at 30.7 MHz. Experimental (●) and calculated (□) ^2H quadrupolar spin-lattice relaxation times (T_{1Q}) as a function of β (angle between H_o and bilayer normal). (*A*) Three-site jump ($\tau_c = 6.7*10^{-10}$ s; fast limit, $\omega_o\tau_c \ll 1$) with site populations 0.46, 0.34, and 0.20 plus axial rotation about the molecular long axis ($\tau_c = 3.0*10^{-7}$ s; slow limit, $\omega_o\tau_c \gg 1$). (*B*) As in *A*, but with increased axial rotation rate ($\tau_c = 8.3*10^{-9}$ s; close to T_1 minimum, $\omega_o\tau_c \approx 0.65$). (*C*) As in *B*, plus effects of Maier–Saupe averaging, $S_{mol} = 0.65$. Replication of data was reproducible within 2%. Experimental error was estimated to be ±5%.

the L_α phase ($S_{mol} = 0.65$) (Jarrell et al., 1987*b*), suggesting that there is less restricted motion in the L_α phase. It is reasonable to assume that the motions used for the gel phase are correct; large angle jumps and/or diffusion about the molecular long axis are the two motions used most commonly to describe lipid motions in bilayers (Blume et al., 1982; Meier et al., 1986; Siminovitch et al., 1985; Wittebort et al., 1987; Dammers et al., 1988; Hauser et al., 1988; Vold and Vold, 1988; Speyer et al., 1989). Obvious modifications to the simulation parameters would be to adjust the rates of the motions already defined.

Modification of the three-site jump rate does not attenuate the anisotropy ($\partial T_1(\beta)$) of the relaxation times; only the absolute T_1 values are affected. This is observed for the simulations of both the $T_1(\beta)$ relaxation profiles and the partially relaxed powder spectra. However, increasing the axial rotation rate by two orders of magnitude ($\tau_c = 8.3 \times 10^{-9}$ s; near the T_1 minimum, $\omega_o\tau_c \approx 0.65$) did affect the anisotropies of the relaxation times ($T_1(\beta)$ relaxation profiles [Figs. 3 *B* (T_{1Z}) and 4 *B* (T_{1Q}) at 30.7 MHz; 46.7 MHz results not shown]) and improved agreement between experimental and simulated partially relaxed T_{1Z} spectra (results not shown). Of particular interest is that the attenuation of the anisotropy ($\partial T_1(\beta)$) is most dramatic for the T_{1Q} profile (Fig. 4 *B*).

Even though adjustment of the axial rotation rate did

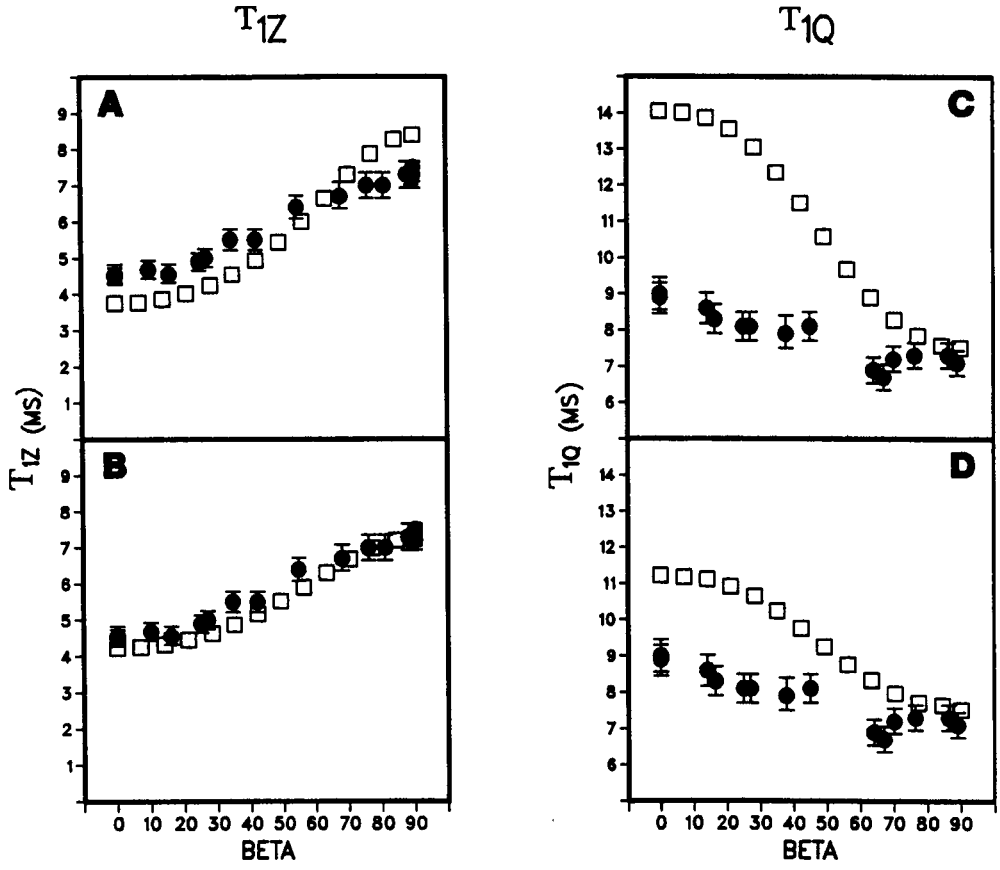

FIGURE 5 Angle-dependent T_{1Z} and T_{1Q} profiles, oriented multibilayers of [3,3-$^2H_2$] β-DTGL at 46.1 MHz. Experimental (●) and calculated (□) ^{2}H spin-lattice (T_{1Z}, *A* and *B*; T_{1Q}, *C*, and *D*) relaxation times as a function of β (angle between H_o and bilayer normal). Simulation parameters as in Fig. 3 *C*, where all three proposed motions are used: three-site jump ($\tau_c = 6.7*10^{-10}$ s; fast limit, $\omega_o\tau_c \ll 1$) with site populations 0.46, 0.34, and 0.20, axial rotation about the molecular long axis ($\tau_c = 8.3*10^{-9}$ s; close to T_1 minimum, $\omega_o\tau_c \approx 0.65$), and effects of orientational averaging, $S_{mol} = 0.65$. Replication of data was reproducible within 2%. Experimental error was estimated to be ±5%.

improve the match of the simulated to the experimental $T_1(\beta)$ relaxation profiles and partially relaxed T_{1Z} spectra, it is apparent that additional averaging is required to describe adequately the type of motion that must be occurring in the L_α phase of [3,3-$^2H_2$] β-DTGL.

Additional averaging in the L_α phase

In the L_α phase there is a distribution of orientations, β'' ($-90° \leq \beta'' \leq +90°$), which the molecular long axis makes with the local bilayer normal (n) (Fig. 2). The orientational distribution, $f(\beta'')$, can be modeled using a Maier–Saupe restoring potential (Van De Ven and Levine, 1984) as

$$f(\beta'') = A \exp [U(\beta'')/kT], \quad (1)$$

where A is a normalization constant, and k and T have their usual meanings. Assuming axially symmetric ordering, $U(\beta'')$ is given by

$$U(\beta'') = [\lambda P_2(\cos\beta'')], \quad (2)$$

where λ determines molecular ordering and can be calculated from the experimental value of S_{mol} by iteratively varying λ until the calculated value of the order parameter agrees with experiment according to

$$S_{mol} = \oint P_2 \cos(\beta'') f(\beta'') \sin(\beta'') \, d\beta''/[\oint f(\beta'') \sin(\beta'') \, d\beta'']. \quad (3)$$

The T_{1Z} values in both the gel and L_α phases of [3,3-$^2H_2$]β-DTGL are similar, indicating that additional averaging in the L_α phase does not significantly influence spin-lattice relaxation. This averaging accounts for the decreased ordering ($S_{mol} \approx 1 \rightarrow S_{mol} = 0.65$) in the L_α phase as compared with the gel phase. The above-

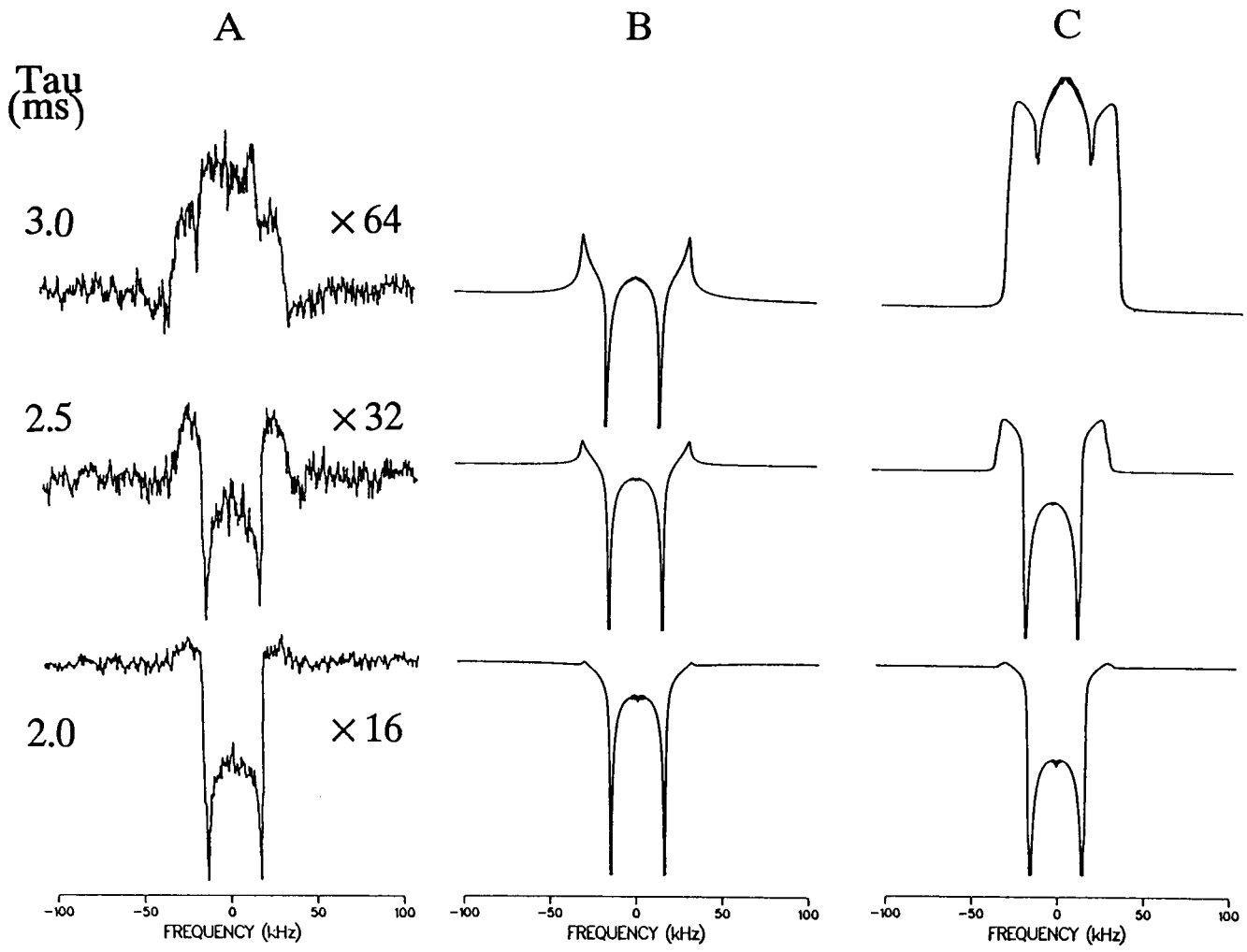

FIGURE 6 T_{1Z} partially recovered powder spectra of [3,3-$^2H_2$] β-DTGL at 30.7 MHz. (*A*) Experimental spectra. (*B*) Simulation of three-site jump ($\tau_c = 6.7*10^{-10}$ s; fast limit, $\omega_o\tau_c \ll 1$) with site populations 0.46, 0.34, and 0.20 plus axial rotation about the molecular long axis ($\tau_c = 1.0*10^{-6}$ s; slow limit $\omega_o\tau_c \ll 1$). (*C*) Three-site jump ($\tau_c = 6.7*10^{-10}$ s; fast limit, $\omega_o\tau_c \ll 1$) with site populations 0.46, 0.34 and 0.20, increased axial rotation about the molecular long axis ($\tau_c = 8.3*10^{-9}$ s; near to T_1 minimum, $\omega_o\tau_c \approx 0.65$), and effects of orientational averaging ($S_{mol} = 0.65$). Tau is the delay between the π and $\pi/2$ pulses in the inversion–recovery experiment.

mentioned fluctuations about the local bilayer normal would lead to orientational averaging of the relaxation rate relative to that expected for a single molecular orientation given only by the orientation of the local bilayer normal relative to the applied magnetic field direction (H_o), β. Thus, for a given orientation β of the local bilayer normal, the orientationally averaged relaxation rate would be

$$\langle T_1^{-1}(\beta)\rangle = \sum_{\beta''=-\pi_{12}}^{\pi/2} T_1^{-1}(\beta') \cdot f(\beta''), \qquad (4)$$

where an orientation of the molecular long axis, β'' relative to the local bilayer normal, leads to its having an associated orientation, β', relative to the magnetic field direction and a corresponding relaxation rate.

For the simulations, the distribution probability of molecular orientations about the order director ($f(\beta'')$) may be determined from the molecular order parameter (S_{mol}) (Van De Ven and Levine, 1984; Vold and Vold, 1988). In the limits of $S_{mol} = 1$ and 0, T_{1Q} and T_{1Z} will exhibit no and complete orientational averaging, respectively.

Application of $T_1(\beta)$ orientational averaging to L_α [3,3-$^2H_2$] β-DTGL with $S_{mol} = 0.65$ results in an attenuation of simulated $T_{1Z}(\beta)$ relaxation profile anisotropies (Figs. 3 *C* [30.7 MHz] and 5 *B* [46.1 MHz]) and leads to a

close correspondence with the experimental profiles. Simulation of partially relaxed inversion recovery spectra also agree very well with the experimental data (Figs. 6 *C* [30.7 MHz] and 7 *B* [46.1 MHz]). To obtain better fits for the partially recovered spectra at 30.7 MHz only, the τ values (delay between the π and $\pi/2$ pulses) had to be lengthened relative to the experimental values (but still within the limits of experimental error); simulated τ values are 2.5, 3.5, and 4.2 ms and the experimental values are 2.0, 2.5, and 3.0 ms, respectively. The sensitivity of the simulated spectra for a given $T_1(\beta)$ profile to the τ value is not unexpected since T_{1Z} is very short. The spectra near the null point will change very quickly as a function of τ; if the simulated T_{1Z} differs from the experimental value, even within experimental error, the spectra will appear to approach equilibrium at different rates. This effect is most obvious around the null point (if T_{1Z} is 4.0 ms the null point is 2.8 ms, but for a T_{1Z} of 4.4

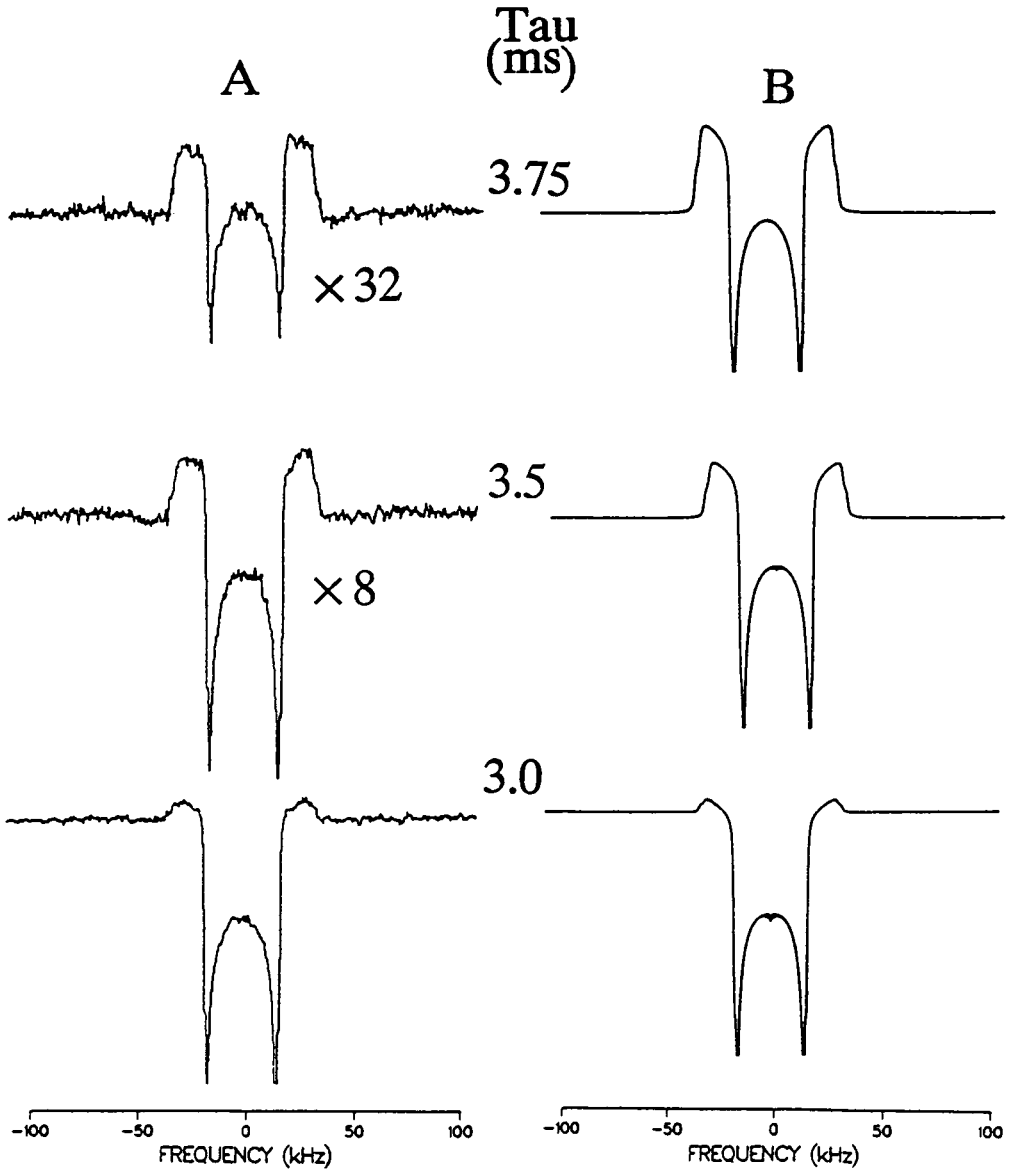

FIGURE 7 T_{1Z} partially recovered powder spectra of [3,3-$^2H_2$] β-DTGL at 46.1 MHz. (*A*) Experiment. (*B*) As in Fig. 5 *B*, where all three proposed motions are modeled: three-site jump ($\tau_c = 6.7*10^{-10}$ s; fast limit, $\omega_o\tau_c \ll 1$) with site populations 0.46, 0.34, and 0.20, axial rotation about the molecular long axis ($\tau_c = 8.3*10^9$ s; close to T_1 minimum, $\omega_o\tau_c \approx 0.65$), and effects of orientational averaging, $S_{mol} = 0.65$. Tau is the delay between the π and $\pi/2$ pulses in the inversion–recovery experiment.

ms the null point is 3.2 ms). For this reason simulation of experimental line shapes using experimental parameters is difficult. It is also important to note that although the absolute τ values are longer than their experimental counterparts, the relative anisotropies in the simulated spectra are consistent with those observed experimentally, which is the most important constraint in the line shape simulation.

There is also a significant attenuation of the simulated $T_{1Q}(\beta)$ profiles when the effects of orientational averaging are included in the calculations. Of the two frequencies used, the fit of the $T_{1Q}(\beta)$ profile at 30.7 MHz (Fig. 4 *C*) is better than that for 46.1 MHz (Fig. 5 *D*). This may reflect the sensitivity of the simulation to the axial rotation rate because the correlation time ($\tau_c = 8.3 \times 10^{-9}$) is closer to the T_1 minimum at 30.7 MHz than at 46.1 MHz ($\omega_0\tau_c = 1.6$ and 2.4, respectively). It is possible to "fine tune" the simulation parameters, but such a small discrepancy in the T_{1Q} profiles is not significant. A factor of 2 attenuation of the rotation rate essentially alters the T_{1Q} by a factor of 2, whereas the effect on the T_{1Z} simulation is much smaller. At this point, the physical meaning of such small changes in the rates of motion remains to be established; the important feature is the profile shape rather than the absolute values. Hence, no further modifications of the simulations have been attempted to improve the simulated orientation-dependent T_{1Q} profiles.

Comments on oriented samples and the T_{1Q} experiment

The insensitivity of the partially relaxed powder spectra to changes in the axial rotation rate is significant and justifies the use of oriented samples. It has been demonstrated recently (Pope et al., 1982; Baenziger et al., 1988; Auger et al., 1990; Bonmatin et al., 1990; Mayer et al., 1990) that oriented samples, as compared with powder samples, allow orientation-dependent effects to be detected unambiguously and are particularly effective for measuring anisotropic relaxation quantitatively. One reason for this difference is that the powder ^{2}H spectrum is a superposition of spectra for which the order director is at random angles (β) to the applied magnetic field. For a C-^{2}H bond executing axially symmetric motions, the observed quadrupolar splitting is given by

$$\Delta\nu_Q(\beta) = (3/8h)\, e^2qQ\, (S_{mol} \cdot \overline{\langle 3\cos^2\theta - 1\rangle})(3\cos^2\beta - 1). \quad (5)$$

e^2qQ is the quadrupolar coupling constant, S_{mol} is the segmental order parameter, and θ is the angle between the order director and the C-^{2}H bond (Fig. 2). In the case of a powder spectrum, spectral contributions for β values of 35° and 90° overlap so that only the weighted average relaxation rates ($(\overline{T_1(90)}^{-1})$) for these orientations can be measured from the spectra directly, $|\Delta\nu_Q(35)|$ and $|\Delta\nu_Q(90)|$ will be equal and their contributions will be superimposed in a powder spectrum. Therefore, any observed T_1 anisotropy, $\partial T_1(B)^{-1}$, will not be $T_1(0)^{-1} - T_1(90)^{-1}$ but rather $T_1(0)^{-1} - \overline{T_1(90)}^{-1}$. With an oriented sample only one β orientation is observed at a time (as set by the experimenter), enabling the unambiguous determination of $\partial T_1(\beta)$.

The $T_{1Q}(\beta)$ relaxation profiles also appear to be more sensitive to changes in the simulation parameters than the $T_{1Z}(\beta)$ relaxation profiles. The significance of this difference and utility of T_{1Q} in testing motional models can be assessed from the definition of the resulting relaxation rates in terms of spectral density functions (Jacobsen et al., 1976*b*):

$$1/T_{1Z} = K[J_1(\omega_0) + 4J_2(2\omega_0)] \quad (6)$$

$$1/T_{1Q} = K[3J_1(\omega_0)], \quad (7)$$

where $K = \{[3/80] \times [e^2qQ/hI(2I-1)]^2\}$, and e^2qQ, h, and I have their usual meanings. In Eqs. 6 and 7 the spectral density functions ($J_m(m\omega_0)$) have a dependence on the orientation of the motional axis relative to the magnetic field direction. The precise nature of this angular dependence relies on the details of the molecular reorientation (Jacobsen et al., 1976*b*; Torchia and Szabo, 1982). Since $J_1(\omega_0)$ and $J_2(2\omega_0)$ may have partially opposing angular dependencies, T_{1Z}^{-1} (Eq. 6) can exhibit a weaker orientational dependence than T_{1Q}^{-1} (Eq. 7). This has been demonstrated by Jacobsen et al. (1976*b*) in the general case of restricted anisotropic motion for a spin 1 nucleus:

$$J_1(\omega_0) = J_1(\omega_0)[1 + (5/7)\langle D_{00}^{(2)}\rangle - (12/7)\langle D_{00}^{(4)}\rangle] \quad (8)$$

$$J_2(2\omega_0) = J_2(2\omega_0)[1 - (5\sqrt{2/7})\langle D_{00}^{(2)}\rangle + (3/7)\langle D_{00}^{(4)}\rangle], \quad (9)$$

where the descriptions of both the orientational dependence and the molecular fluctuations are denoted by the Wigner rotation elements in the angular brackets.

This behavior has also been recognized previously by Wefing et al. (1984), who noted that the line shape changes of partially relaxed spectra observed in T_{1Z} experiments may be minor compared with those observable in T_{1Q} experiments. The essential point is that the difference in the angular-dependent behavior of T_{1Z} and T_{1Q} would imply that in oriented spectra (and possibly powder spectra), the Jeener–Broeckaert experiment may be more useful than the inversion–recovery experiment for discriminating between different motional models. This difference in orientational sensitivity is evident upon comparison of Figs. 3 and 4.

The glucose head group

In the preceding section, a description of molecular reorientation of the glycerol C3 segment was elucidated, which is consistent with spin-lattice relaxation measurements in the liquid-crystalline phase. Since the carbohydrate head group is linked directly to the C3 segment, it is natural to explore the use of the dynamics of the C3 fragments to describe the reorientation of the glucose moiety. Previous work (Jarrell et al., 1987*a*) showed that there is probably motion of small amplitude about the glucose–glycerol glycosidic link. As a starting point for this study, this small amplitude motion was ignored and the head group–glycerol segment of β-DTGL was assumed to be rigid with the conformation deduced previously (Jarrell et al., 1987*a*). Thus, the initial isomerization about the glycerol C2-C3 bond, as well as reorientation about the molecular long axis, lead to corresponding reorientation of the assumed rigid head group fragment.

Head group reorientation was investigated with {1-$^2H_1$} β-DTGL in the L_α phase, where the ^2H label is at the C1 position of the glucose ring. The glycolipid was studied as a multilamellar aqueous dispersion. Partially relaxed T_{1Z} spectra are presented in Fig. 8 and clearly reveal anisotropic relaxation. Simulations using the above motional model and rates deduced for the glycerol C3 position predict a larger T_{1Z} anisotropy than is seen experimentally (data not shown). Addition of orientational averaging arising from molecular fluctuations about the bilayer normal ($S_{mol} = 0.65$), in analogy with that used for the glycerol C3 position, attenuated the predicted T_{1Z} anisotropy and greatly improved the correspondence between the simulated and experimental relaxation profiles (Fig. 8). As was the case with the simulated, partially relaxed 30.7-MHz spectra of {3,3-$^2H_2$} β-DTGL, the τ values used for the simulation of {1-$^2H_1$} β-DTGL spectra had to be lengthened to have the simulated, partially relaxed spectra better resemble the experiment (experimental τ values are 3.25 and 3.75 ms; simulated τ values are 3.5 and 4.6 ms, respectively). In addition, one must also be reminded that the T_1 values used are estimated from powder spectra and some error will be associated with $T_1(90)$ in the $T_1(\beta)$ relaxation profile due to spectral overlap associated with β of 35° or 90° (see above). In fact, quick inspection of the simulated $T_1(\beta)$ relaxation profile indicates that $T_1(35) < T_1(90)$. Removal of the estimated $T_1(35)$ contribution to the experiment $T_1(90)$ value would increase $\partial T_1(\beta)$ and improve the fit of simulated to experimental results.

In general, the reorientational model and associated rates elucidated for the glycerol C3 segment also provide a good description of the motion of the glucosyl head group. Close inspection of Fig. 8 reveals that quantitatively the simulated results differ from the experimental results. A potential source of this discrepancy may be the assumption that the glucose–glycerol linkage is rigid. Previous work has shown that for β-DTGL in the L_α phase the head group has a segmental order parameter of 0.45, which is smaller than that of the glycerol segment, 0.65 (Jarrell et al., 1987*b*). The reduced segmental ordering probably reflects torsional fluctuations of the glucose moiety about the glycosidic bond. It is clear from Fig. 8 that such motions must be included to further improve the agreement between predicted and experimental relaxation profiles. We are presently investigating the use of molecular mechanics calculations to define the minimum-energy conformations of the head group about the glycosidic bond with respect to the glycerol backbone. Inclusion of an additional internal jump among these sites will permit the extension of the present study to a more comprehensive description of head group dynamics. Such an approach has been reported recently in a molecular dynamics study (Edge et al., 1990), which calculated the minimum-energy conformations of disaccharide glycosidic linkages found in glycoproteins.

Comparison with previous work

Limited orientational averaging of T_{1Z} has been used to describe the dynamics of egg phosphatidylcholine (Milburn and Jeffrey, 1989). The dynamics of the phosphodiester moiety are described in terms of two motions, a fast axially symmetric rotation and a slower wobble. The slow wobble that averages the T_1 orientation dependence is not unlike the molecular reorientations described above. Although this motional model does fit the data, it is probably an oversimplification of the actual behavior.

The present study has the advantage that preliminary investigations of {3,3-$^2H_2$} β-DTGL in the gel state (Auger et al., 1990) provide a firm basis for describing the L_α dynamics: the molecular motions were refined and a further, more complex motion was identified. The three motions described are physically reasonable and have been used either individually or in combination to describe other lipid systems (Blume et al., 1982; Siminovitch et al., 1985; Meier et al., 1986; Wittebort et al., 1987; Dammers et al., 1988; Hauser et al., 1988; Vold and Vold, 1988; Speyer et al., 1989; Mayer et al., 1990).

Mayer et al. (1990) have elucidated the dynamics of specifically deuterated 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) using T_{1Z} results of oriented multibilayers. They made a comparison of motions in a

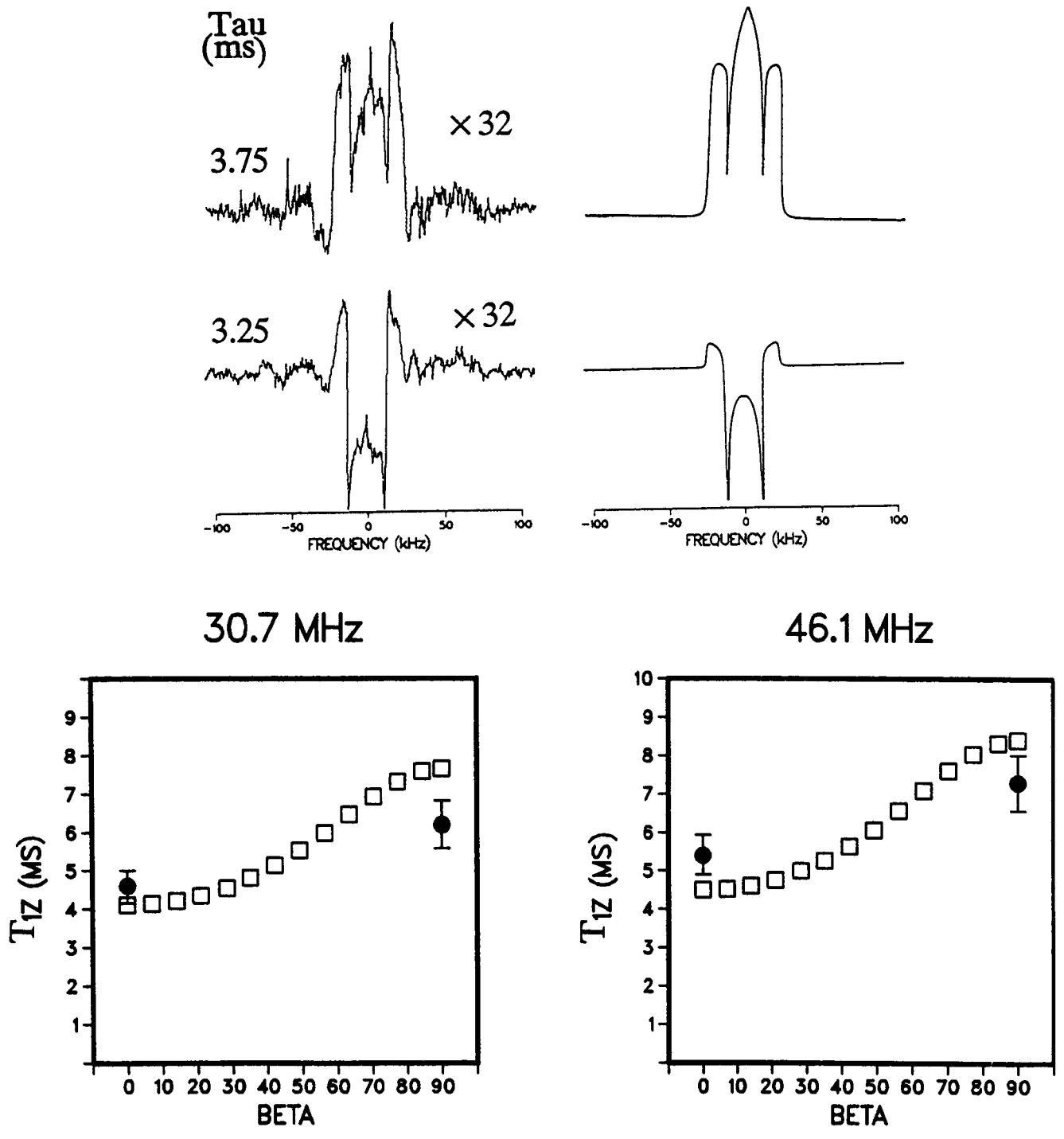

FIGURE 8 (*Top*) T_{1Z} partially recovered powder spectra of $\{1'\text{-}^2H_1\}$ β-DTGL at 30.7 MHz. (*Left*) Experiment. (*Right*) Calculated spectra. Simulation parameters are the same as in Fig. 3 *C*, where all three proposed motions are used: three-site jump ($\tau_c = 6.7*10^{-10}$ s; fast limit, $\omega_o\tau_c \ll 1$) with site populations 0.46, 0.34, and 0.20, axial rotation about the molecular long axis ($\tau_c = 8.3*10^{-9}$ s; close to T_1 minimum, $\omega_o\tau_c \approx 0.65$), and effects of orientational averaging, $S_{mol} = 0.65$. Tau is the delay between the π and π/2 pulses in the inversion–recovery experiment. (*Bottom*) Angle-dependent T_{1Z} profiles from powder spectra of $\{1'\text{-}^2H_1\}$ β-DTGL at 30.7 MHz (*left*) and at 46.1 MHz (*right*). Experimental (●) and calculated (□) ^2H spin-lattice relaxation times (T_{1Z} only) as a function of β (angle between H_o and bilayer normal). Simulation parameters are the same as the above spectra. T_{1Z} values are estimated from powder spectra; therefore, error is ±10%.

restricted state (cholesterol present) and a less restricted state (pure DMPC), similar to our comparison of gel and liquid-crystalline state β-DTGL. Furthermore, the three basic motions are very similar to those used in the present analysis; internal isomerization (about four sites, versus three), rotation about the molecular long axis, and orientational averaging arising from fluctuations about the bilayer normal. The essential difference in the simulation of dynamic behavior of the restricted and less restricted DMPC states is the rate of the motions and, most importantly, the effect of the molecular fluctuations, as reflected by the segmental order parameter (the simulations used a population weighted potential, similar to the Maier–Saupe potential used here). The rates of motion used for both states are within an order of magnitude of those used here (as expected since motions around the time frame of the Larmor frequency most affect relaxation). The major difference between the present and the latter studies is that this one has not assigned a correlation time to the orientational averaging process because we assume that these fluctuations are too slow to affect relaxation.

Conclusions

It has been possible to apply a motional model deduced for the glycerol C3 position of β-DTGL in the gel state not only to the more fluid L_α phase, but also to the analysis of the glucosyl head group ($[1'\text{-}^2H_1]$) motions of β-DTGL in the L_α phase. This translation of modeled motion from the glycerol backbone of the head group is intended to describe the basic "correlated" head group motions with the entire β-DTGL molecule. Work is in progress to complete the description of the more complex motion of the carbohydrate group, which is considered to be independent of the other molecular motions, torsional motions about the glycosidic bond. It should be noted that the basic features of spin-lattice relaxation have been reproduced using discrete values for the rates of molecular motion. While distributions of motional rates for each of the two motions might lead to improved fits between predicted and observed relaxation profiles, their inclusion would not change the essential conclusions of the present study.

This study further demonstrates the necessity of using both oriented samples and T_{1Q} experiments in testing the motional models, in addition to the well-known multiple frequency approach. Oriented multibilayers not only avoid the effects of fast lateral diffusion over the curved vesicles, which may average completely anisotropic spin-lattice relaxation (Brown and Davis, 1981), but they also provide a means to determine unambiguously the spin-lattice $T_1(\beta)$ relaxation profiles in detail. Moreover, the $T_1(\beta)$ profiles will identify more accurately features associated with a particular motional model. Notably, the T_{1Q} experiment has been useful in testing further the molecular models described. The sensitivity of the $T_{1Q}(\beta)$ relaxation profile to changes in the simulation parameters has proven that T_{1Z} spin-lattice relaxation data alone may not be sufficient to discriminate between models for the more detailed molecular motions.

In effect the additional battery of tests (two frequencies, oriented multibilayers, powder spectra line shapes, and both T_{1Z} and T_{1Q} experiments) have outlined the complex motions of β-DTGL in its biologically relevant liquid-crystalline state. While this study has focused on a simple glycolipid, it suggests that the methodology used may assist in unraveling the conformational and motional aspects of more complex glycolipids in a membranous environment

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